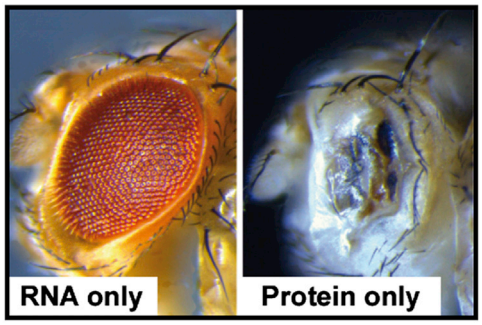
Cell

An RNA Nexus in Neurodegeneration

The causes of neuronal death in neurodegenerative diseases are multifaceted, but an emerging theme in a number of disorders is the role of RNA regulation and dynamics. In amyotrophic lateral sclerosis (ALS) and frontotemporal dementia, RNAs arising from an expanded GGGGCC repeat are thought central to the pathogenic process, with recent data revealing why and offering a new strategy by which the deleterious RNA might be directly targeted. Likewise, a compelling potential therapeutic avenue for spinal muscular atrophy is illuminated by a new study in which small molecules are shown to selectively control alternative splicing of the *survival of motor neuron 2* gene and ameliorate disease symptoms in mice.



Toxicity in the *Drosophila* eye is caused by "protein-only" poly (Gly-Arg) repeats, but not by "RNA-only" GGGGCC repeats of equivalent length. Image courtesy of S. Grönke.

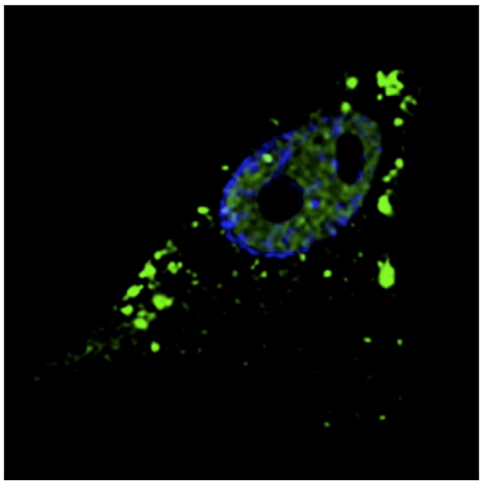
Dangerous Duplicated Dipeptides

Why do expanded nucleotide repeats have toxic effects in many neurodegenerative diseases? New findings by two groups point to the possibility in frontotemporal dementia and amyotrophic lateral sclerosis (ALS) that primary culpability comes from translation products that arise from an expanded GGGGCC repeat in C9orf72, the most prevalent genetic lesion in the two related disorders. It is known that the GGGGCC repeat RNA forms aggregates in neurons and that the RNAs can also encode proteins with dipeptide repeats (which are translated in a manner that is not dependent on ATG start codons), but it has not been clear whether toxicity is mediated by the RNAs (sense and antisense), or proteins, or both. Miezelinska et al. (2014) inducibly express an extended GGGGCC repeat construct or a construct with periodic stop codons inserted in adult fruit flies. Both RNAs form foci in vivo, both fold in vitro into G-quadruplexes (the tertiary structure formed by GGGGCC repeats), but only the one with the potential to encode dipeptide repeat proteins leads to neurodegeneration. Taking into account both the sense and antisense strands, the GGGGCC expansions encode five different dipeptide repeat proteins, and by incorporating alternate codons to change the RNA secondary structures, the authors provide evidence that it is the peptides specifically containing arginines (Gly-Arg and Pro-Arg) that are deleterious.

Working in cultured mammalian cells, Kwon et al. (2014) show that these arginine containing repeat peptides bind to nucleoli and cause cell death by impeding RNA biogenesis. Interestingly, arginine repeats (specifically serine-arginine) are common in proteins that control pre-mRNA splicing, and the authors show that the serine-arginine (SR) repeat of SRSF2 binds to hydrogels containing the low-complexity domain of heterogeneous ribonucleoprotein A2 and in vivo the SR repeat associates with nucleoli. Its binding is reversed when the proteins become phosphorylated by the CDC2-like kinases 1 and 2 (CLK1/2), whereas the binding of Gly-Arg and Pro-Arg repeat proteins, as relevant to C9orf72 expansion disorders, is not countered by CLK1/2. Consistent with this observation, variants of SRSF2 with serines mutated to glycines can no longer be liberated from nucleoli by CLK1/2. These findings argue that the cellular pathology of ALS and frontotemporal demensia could be closely related to native processes of regulated protein aggregation that control ribosomal RNA biogenesis and splicing. Compellingly, the authors show that the presence of Gly-Arg and Pro-Arg repeat peptides alters splicing of the amino acid transporter EAAT2 in a manner identical to that observed in ALS. Another surprising discovery is that the repeat peptides are readily taken up by cells in culture and translocate to the nucleus. The authors pose the interesting notion that the release of these peptides upon the cell death could contribute to the spread of pathology to neighboring cells. Could some kind of snowball effect related to this process underlie the nefarious course of the diseases, and if so what can be done to slow or stop it?

Kwon, I., et al. (2014). Science. Published online July 31, 2014. http://dx.doi.org/10.1126/science.1254917.
Mizielinska, S., et al. (2014). Science. Published online August 7, 2014. http://dx.doi.org/10.1126/science.1256800.

Small Molecule Gets to the Crux of a Hexanucleotide Repeat Disorder



Poly (Gly-Pro) proteins are observed in neurons derived from fibroblasts with the C9ORF72 repeat expansion.

Regardless of whether toxicity in frontotemporal dementia and ALS arising from the C9orf72 expansion is primarily due to RNA foci formation or to accumulation of dipeptide repeat translation products, targeting the RNA structure could be therapeutically beneficial. This premise inspired the efforts of Su et al. (2014), who have now created a small molecule that will target GGGGCC RNA expansions. By screening molecules chemically similar to previously identified CGG RNA expansion binders, the authors find compounds that stabilize and/or bind hairpins and quadruplexes formed by GGGGCC expansions. They then test these compounds for their capacity to reduce RNA foci formation and inhibit translation of the dipeptide repeat containing proteins. These products are known as c9RAN proteins with RAN being an acronym for repeat associated non-ATG translation, reflecting the fact that their translation does not initiate at a canonical start codon. This winnowed the list of candidates down to one lead compound, known as 1a, which is shown to ameliorate expression of poly (Gly-Pro) from the sense strand, but not impact poly (Gly-Pro) or poly (Pro-Arg) from the antisense strand. 1a and another compound block RAN translation and foci formation in neurons derived from fibroblasts with the expansion. In an additional step toward clinical exploration, the authors show that poly (Gly-Pro) in cerebrospinal fluid is a biomarker of ALS patients with the C9orf72 expansion. This raises the possibility that RAN products could one day be used in clinical trials to ascertain the efficacy of drugs targeting GGGGCC expansions. The recent findings (discussed above) that arginine containing RAN products may be key to disease pathogenesis should motivate efforts to also target the antisense strand. That future effort might be modeled on the thoughtful and successful approach employed by Su and colleagues.

Su, Z., et al. (2014). Neuron. Published online August 14, 2014. http://dx.doi.org/10.1016/j.neuron.2014.07.041.

Drugging Splicing in SMA

Spinal muscular atrophy, characterized by progressive motoneuron loss and weakness, is the most common genetic cause of infant mortality. It is a recessive disorder caused by the mutation or loss of the *SMN1* (*survival of motor neuron 1*) gene. Although its paralogue *SMN2* is ubiquitously expressed, a translationally silent C-T transition in exon 7 impacts its alternative splicing, favoring the exclusion of exon 7, and as a result only a fraction of the *SMN2* produces the full-length SMN protein and thus only partially compensates for the loss of *SMN1*. Enter Naryshkin et al. (2014) who, by screening a library of small molecules against a cell line carrying an *SMN2* minigene, identify small molecules that promote inclusion of exon 7 into mature *SMN2* mRNA. The initial hits were then optimized by medicinal chemistry. Similarly impressive results are obtained for the compounds when tested in motoneurons derived from induced pluripotent stem cells generated from SMA patient-derived fibroblasts. Not only is inclusion of exon 7 increasingly favored, SMN protein levels are substantially increased and, surprisingly, the splicing of very few other genes are impacted. In mouse models of SMA these compounds promote increased SMN protein levels, improved motor function, and increased longevity. The challenges now will be to determine how these compounds act, perhaps with an eye toward making the compounds even more selective and efficacious, and to drive the work forward to a potential therapy. It is encouraging that a compound from this group, RG7800, has recently passed through stage 1 safety testing in healthy adults in whom increased production of full-length *SMN2* mRNA is also observed.

Naryshkin, N.A. (2014). Science. 345, 688–693.

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